## **CLAIMS**

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## 1. A protease variant comprising:

a) an insertion, substitution or deletion of one of the amino acid residues K,H,R,E,D,Q,N,C,V,L,I,P,M,F,W,Y,G,A,S,T in one or more of the positions 62, 68, 97, 98, 99, 106, 131, 170, 245, 252,

in combination with at least one of the following modifications

\*0AQSVPWG; A1T,V; Q2L; S3T,A,L; V4L,A; I8V,T; S9G,D,R,K,L,V; R10H,K; V11A; Q12D; A13V; P14S,T,D,A,M,V,K,Q,L,H,R,I; A15M,T; A16P; H17R; N18S,H; R19W,K,L,F,G,I; G20\*,R,A; L21F,LP,LW,LA,LG; T22S,A,K,TV,TG,TL,TW,TV,G,L,TY; G23S; S24P; K27R, V28I; V30I; I35T,V;T38S; P40L; N43D; R45H,K; G46D; A48T; S49N; F50S; V51A,I,D; P52V,A; P55S,A; S57P; G61E,D,S,R,GP; N62D,ND,NE,DE,NG,E,S; V68A,S,L,I; T71A; I72V; L75I; N76S,D; N77S; S78T; V81A; A85T; S87C; A88V,T; E89G; K94N; V95C,T; L96LA,LG; G97E,D,W,A,GG,GA,GV,N,GS; A98S,D,E,T,AS,AD,AV,AE,AH,Q,N,M,L,G,R,V,S; S99D,L,A,AD,SD,SM,SG,DA,P,G,N,C,M,V,I; G100S,GE,C; S101SA, SK; G102D,S; S103D,E,Y,L,Q,H,T; V104T,S,R,I,N,M,L,D; S106D,E,T,M,G,A,L,F,I; I107T,V,M; A108V,T,S;

L111I,V; A114V; N116S,D; G118D; M119L,I,V,A,S; H120N,D,Q,K,E,Y,S; V121A; L124C; L126I; G127E; S128N,I,G,C; P129PSN,T,E,D,S,N,A; S130P,T,C,\*; P131M,F,W,L,A,H,T,\*,PA,S,Q,R,E,G,D,C; S132G,T; A133ASA; T134A; Q137H,E,D; A138G,V; V139L,I; N140D, K; T143A; S144D, N,P; R145G; V150I; A151V,G; A152P; A158T,V,C,E,L,D, M; G160A,D; S163G,C,N,A; Y167K,A,I; A168G; A169G; R170C,S,H,L; Y171C; A172V; N173D; A174V; M175L,I,V,A,S,T; N183D; N184D,S; N185S,D; R186L,C,H; S188G; S190A; Y192H;

G195F,E; V203S,A,L,Q,M, F,I; N204T,D,S; Q206L; Y209C,H; G211D; S212N,L; T213A; Y214C,H; A215D,T; N218D,S; M222L,I,V,A,S; A223G; T224A,S; A228T; A230V; A232S,L,T,P; V234I; Q236A,L,D,T,C,M,F,S; K237R; N238D; P239T,S; S240F; S242T; V244I,M,A;

Q245R,K,E,D,T,F,N,V,W,G,I,S,C,L,A,M; N248P,D,S; K251E,R; N252G,H,D,V,M,S,T,E,Y,S,Q,K,A,L; A254S; T255A,S; S256N,R,G; L257G; G258K, S259A,N,G; T260A,R; N261D; L262S, Q,V; Y263H,F; G264E; S265G,R,N; V268L,I; N269T; N296K; E271A; T274S,L,A,R, or

b) one of the following combination variants

A108T+L111V; L124I+S125A; P129S+S130AT; L96LA+A151G+V203A; S49N+V203L+N218D; S3T+A16P+R45C+G100S+A230V; I8V+R19K+V139I; N76D+A174AL+A194P+A230V; N185R; N62NE; H120Q+Q137E, G61GE, G61GS, G100L, A133D, V68A, N123D, L111F+Y263H, V11A+G61GE+V227A+S240F, A133E+S144K+N218D, S128A+P129S+S130SP,

G97E. Q245W. S9R+A15T+T22TQ+S101P, S9R+A15T+H120R+Q137D+N173S, S9R+A15T+L111V+Q137E+G211D, S9R+A15T+L96LG+Q137E+Y209H, S9R+A15T+L111I+H120N+Q137E, S9R+A15T+L111I+Q137E. S9R+A15T. S9R+A15T+L96LG+H120Q+Q137E, S9R+A15T+T260M. Q2451, S9R+A15T+S130P. Q245F. S9R+A15T+N218D, S9R+A15T+H120G+Q137E+N218D. G100S. S9R+A15T+D60DG. G63E+N76D+A194P+A230V, S9R+A15T+T224A, A108V+A169G+R170A+Y171H, A138V+V139I+A194P+N218D+A230V, I8V+P14L+R19L+V30I+I35V+S57P+P129S+Q137D+S144D+S256N, A133D+T134S+Q137A, Q137D, A98AH, V51D, Q12E+P14L+A15T, G63E+N76D+A194P+A230V, Q12E+P14L+A15T, G97GS, V51A+S163T; V139I+A151G; S9R+A15T+L96LG+S130\*; A169G+R170H or

- c) one or more modifications in position 68, wherein said modification(s) comprise(s): deletion, insertion and/or substitution of an amino acid residue selected from the group consisting of K,H,R,E,D,Q,N,C,V,L,I,P,M,F,W,Y,G,A,S and T.
- 15 2. A protease variant of claim 1 comprising the combination of one or more of the modifications

X62D,E,S,XD,XE,XG,DE

X68A,S,L,I

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X97E,D,W,A,N,XG,XA,XV,XS

X98S,D,E,T,N,M,L,G,R,V,XS,XD,XV

X99D,L;A,P,G,N,C,M,V,I,AD,XD,XM,XG,DA

X106D,E,T,M,G,A,L,F,I

X131M,F,W,L,A,H,T,\*,S,Q,R,E,G,D,C,XA

X170C,S,H,L

X245R,K,E,D,T,F,N,V,W,G,I,S,C,L,A,M

X252G,H,D,V,M,S,T,E,Y,S,Q,K,A,L

with at least one of the following modifications

\*0AQSVPWG; A1T,V; Q2L; S3T,A,L; V4L,A; I8V,T; S9G,D,R,K,L,V; R10H,K; V11A; Q12D; A13V; P14S,T,D,A,M,V,K,Q,L,H,R,I; A15M,T; A16P; H17R; N18S,H; R19W,K,L,F,G,I; G20\*,R,A; L21F,LP,LW,LA,LG; T22S,A,K,TV,TG,TL,TW,TV,G,L,TY; G23S; S24P; K27R, V28I; V30I; I35T,V;T38S; P40L; N43D; R45H,K; G46D; A48T; S49N; F50S; V51A,I,D; P52V,A; P55S,A; S57P; G61E,D,S,R,GP; N62D,ND,NE,DE,NG,E,S; V68A,S,L,I; T71A; I72V; L75I; N76S,D; N77S; S78T; V81A; A85T; S87C; A88V,T; E89G; K94N; V95C,T; L96LA,LG; G97E,D,W,A,GG,GA,GV,N,GS; A98S,D,E,T,AS,AD,AV,AE,AH,Q,N,M,L,G,R,V,S;

S99D,L,A,AD,SD,SM,SG,DA,P,G,N,C,M,V,I; G100S,GE,C; S101SA, SK; G102D,S: S103D,E,Y,L,Q,H,T; V104T,S,R,I,N,M,L,D; S106D,E,T,M,G,A,L,F,I; I107T,V,M; A108V,T,S; L111I,V; A114V; N116S,D; G118D; M119L,I,V,A,S; H120N,D,Q,K,E,Y,S; V121A; L124C; L126I; G127E; S128N,I,G,C; P129PSN,T,E,D,S,N,A; S130P.T.C.\*; -P131M,F,W,L,A,H,T,\*,PA,S,Q,R,E,G,D,C; S132G,T; A133ASA; T134A; Q137H,E,D; A138G,V; V139L,I; N140D, K; T143A; S144D, N,P; R145G; V150I; A151V,G; A152P; A158T,V,C,E,L,D, M; G160A,D; S163G,C,N,A; Y167K,A,I; A168G; A169G; R170C,S,H,L; Y171C; A172V; N173D; A174V; M175L,I,V,A,S,T; N183D; N184D,S; N185S,D; R186L,C,H; S188G; S190A; Y192H; G195F,E; V203S,A,L,Q,M, F,I; N204T,D,S; Q206L; Y209C,H; G211D; S212N,L; T213A; 10 Y214C,H; A215D,T; N218D,S; M222L,I,V,A,S; A223G; T224A,S; A228T; A230V; A232S,L,T,P; V234I; Q236A,L,D,T,C,M,F,S; K237R; N238D; P239T,S; S240F; S242T; V244I,M,A; Q245R,K,E,D,T,F,N,V,W,G,I,S,C,L,A,M; N248P,D,S; K251E,R; N252G,H,D,V,M,S,T,E,Y,S,Q,K,A,L; A254S; T255A,S; S256N,R,G; L257G: G258K, S259A,N,G; T260A,R; N261D; L262S, Q,V; Y263H,F; G264E; S265G,R,N; V268L,I; N269T; 15 N296K; E271A; T274S,L,A,R.

## 3. The protease variant of claim 1, comprising one or more of the following alterations:

G97E+A98S	V28I+A98AD+T224S
G97D+A98D	S99AD+M175V+P131F
V95C+G97W+A98E	S99AD+P131L
V95T+G97A+A98D	S9R+S99AD+P131W
S103Y+V104M+S106D	V68A+N116S+V139L+Q245R
V104T+S106D	S3T+A16P+R45C+G100S+A230V
S3T+A16P+S99SD+S144D+A158T+	I8V+S9R+A15T+R19W+V30I+G61D+
A230V+T260R	S99SD+S256N
S103D+V104T+S106T	V30I+S99SD+S256R
S103D+V104L+S106M	G61S+S99SD+V244I
S103D+V104T+S106G	V68A+V139L+S163G+N185S
S103D+V104S+S106A	S99SD+Y263H

S103H+V104N+S106D	V104N+S106T
S103E+V104I+S106T	S99SG+S144D
S103Q+V104T+S106E	V30I+S99SD
S103E+S106T	N18H+S99SD
S103E+V104R+S106A	S9R+T22S+S99SD+K251E
A108T+L111V	A48T+V68A+P131M
L124I+S125A	A15M+S99SM+V139I+V244I
L124C+P131*	P14T+A15M+S99SD
P129S+S130AT	I8V+S99SD+S144D+A228T
L96LA+A151G+V203A	I8V+R19K+V139I
S99SD+A108V+V139L	135T+N62D
S99SD+S190A	N62D+S265G
S99SD+V203A	Q2L+N62D
S99SD+V139I	N62D+N76D
S99SD+A108V	R45H+G61E+V68A
S99SD+S106A+A151G	N62D+V121A
V68A+S106A	N62D+A215D
V68A+N185D+V203S	N62D+N238D
V68A+V139L	N62D+R145G
V68A+V139I	V4L+N62D+E89G
V68A+A158V	N62D+S188G+K251R
V68A+V203A	S49N+N62D
V68A+V203S	N62NE
V68A+V203L+S259A	V11A+N62DE

V68A+S106L	N62ND+N184S+S256G
V30I+V68A+V203S	N18S+N62D+I107T+A254S
V51A+V68A+S106T+A168G	S57P+N62ND
V51A+V68A+S106T+A168G	N62NE+V234I
V68A+N76S+V203M+P239T	Q137H+R170C+G195E
V68A+V203L	S99A+S101SA
V68A+L75I+V203Q	R10K+P14A+R19K+A98AS+S128N
V68A+T71A+V139L	T22A+R45K+A98AS+S128N
Y192H+V68A	A98AV+S99D+Y167K
V68A+S106A+A108T	S9G+P14K+Y167A+R170S
V68A+S106T+A108T	S9D+P14T+Y167A+R170S
V68S+A108S	S9R+P14M+A98AD
V68A+N76S+G211D	S9R+R19L+A98AD+E271A
V68A+S106T+A108T	S9R+P14S+R19F+A98AD
A151V+R170C	S99DA+P129PSN+P131A
P14D+A98AS+H120D+G195F+	S99AD+V244M+Q245K+N248D+
S212N+M222S	K251R+T255A+S256N
S49N+V203L+N218D	S9R+P14V+R19G+A98AD
V68A+S106M+N184D	S99AD+N248P+T255A+S256G
P55S+V68L+A158E+G160A	*0AQSVPWG+A98AD
V68A+A158C	T22A+S99AD
V68A+A158L+Y214C	K94N+A98T+S99L
A88V+S99AD+P131F	N76D+A174AL+A194P+A230V
P14T+A16P+I72V+S99SD+V244I+T260A	P40L+N218D+A232S+Q236L+Q245E+S259N
S99AD+P131F	A232L+Q236D+Q245E
	<u> </u>

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R10H+N62D	A232T+Q236L+Q245D
V28I+A98AD+T224S	R170H+Q236A+Q245R
S9K+T22K+S99AD	A232L+Q236T+Q245D
P14S+S99AD+P131W	G97GG+P131H+Q137E+V268L
V68A+I72V+P131F	A88V+G97GV+P131H
S9R+S99AD	G97GA+H120Q+S130P+G264E
S9K+S99AD	G97GG+V139L
V28I+A88V+G100S+P131M	G97GG+Q137D
S103L+V104S+S106G	G97GG+H120D+Q137H
V68A+T224A	N185R
V68A+P131F	P131H+Q137E
A48T+V68A+P131M	V104I+H120N+P131H+Q137E
V68A+I72V+P131F	H120Q+Q137E
G100GE+P131F	S9R+A15T+G97GV+H120D
S99AD+P131F+T260A	G100S+H120Q+Q137H
R19G+A98AS	V68A+H120K+Q137E
G61R+N62D	G97GA+H120E
V68A+S106M+N184D	H120D+S128I+Q137D
P55S+V68L+A158E+G160A	G97GG+P131H
V68A+A158C	G97GG+H120N+L126I
R19W+G61S+S99SD+N204T+Y263H+	S9R+A15T+G97GA+H120D+P131H+
S265R	Q137E
A232T+Q236C	S9R+A15T+G97GV+P131T+Q137H
N62D+A232T+Q236C	S9R+A15T+G20*+L21F+N62D+Q245N
A232P+Q236L+Q245E	S9L+A15T+T22TV+V139L+Q245F
G61R+N62D V68A+S106M+N184D P55S+V68L+A158E+G160A V68A+A158C R19W+G61S+S99SD+N204T+Y263H+ S265R A232T+Q236C	G97GA+H120E  H120D+S128I+Q137D  G97GG+P131H  G97GG+H120N+L126I  S9R+A15T+G97GA+H120D+P131H+ Q137E  S9R+A15T+G97GV+P131T+Q137H  S9R+A15T+G20*+L21F+N62D+Q245N

A232S+Q236L+Q245T+K251E	S132G+Q245F
S163C+Q236M+Q245T+S256G	S9R+A15T+T22TG+N62D+V139L+Q245V
N218D+A232L+Q236F+Q245F	S9L+A15T+T22TV+V139L+Q245F+L262S
S163N+A232L+Q236S+Q245E	S9R+A15T+T22TL+N62D+Q245W
A232S+Q236S+Q245E	V68A+A158L+Y214C
V68A+V203L	N62D+V150I
V68S+A158D	S3T+P14Q+A15M+R19K+N62D+S144D
I8V+A15T+R19K+A85T+S99SD+A114V+	P14Q+R19W+V51I+G61E+S99SD+
V244I+S256N+Y263H	V139I+T260R
L111F+Y263H	S3T+P14L+H17R+S99SD+V139I+S144D
P52V+S78T+S99SD	S3A+V30I+S99SD+S106G+N248S
A15M+S99SD+V268I	18V+A15T+S99SD
S99G+S128N+N183D+A232L+Q236T+	S3T+S9R+P14H+A15M+R19L+S99SD+
Q245R	V139I
S99R+S101SA	S9R+A15T+G97GG+H120D+Q137E
L96LA+A98T+P131AA	S9R+A15T+G20A+G97GV+H120D+P131H
A98E+S99P	S163N+A232L+Q236A+Q245G
V28I+S99AD+P131F	N173D+A232L+Q236A+Q245N
S9R+A15T+G97GV+Q137H	P55S+V68A+S106M+A108T+P129T
V81A+P131T+A133S+Q137E	K27R+V68L+G118D+A158E
N43D+V68A+S106F+N238D	A98E+S99A+S101SK
V68A+V203F	V68A+N140D+T143A+S144N
V68A+S106E	N62D+N140K+T143A+S144D
V68A+S106I	S9F+P14T+R19L+A98AD
V68A+A158M+R170C	S9V+P14R+R19F+A98AD

	<del></del>
V68A+P129T+N218D	S99A+S99SD+G258K+L262Q
V68S+P129E	S87C+S99SA+S99D+P131A
V68S+P129D	S99A+S99SD+G258K+L262Q
V68L+P129E+N261D	V28I+S99A+*99aD+P131F
G97GV+H120D	A85T+G102D+S106T+K237R
P131A+A133ASA	V68A+T71A
L111F+Y263H	G61GS
V11A+G61GE+V227A+S240F	G100L
A133E+S144K+N218D	A133D
S128A+P129S+S130SP	V68A
G61GE	N123D
S9R+A15T+T22TW+N204D+Q245I	Q245W+N252V
S9R+A15T+G97GG+P131S+Q137H	R45H+Y171C+Q245W+N252S
S9R+A15T+T22TG+N62D+V139L+Q245G	G20R+A48T+R170C+Q245W+N252Q
S9R+A15T+T22TL+N62D+I107V+V139L+ Q245W	S9R+A15T+A16P+G97GA+P131S+Q137D+ N204S
S9G+A15T+G97GA+Q137H	N218D+Q245W+N252E
S9R+A15T+V68A+Q245R	G20R+R170C+Q245R+N252V
S9R+A15T+G97GA+H120N+S212L	S9R+P14I+R19K+A98AD+T274S
S9R+A15T+L96LG+H120D+P131H+R186L	A98AE+V203I
S9R+A15T+G97GA+H120D+Q137D	V51A+V68A+S163G+V203A
N62D+N252T	N62D+Q245W+N252H
V4A+S9R+A15T+G97GV+H120D	N62D+Q245W+N252A
S9R+A15T+G97GV+H120D+Q137H	G20R+N62D+V244I+Q245W+N252E
S9R+A15T+L96LG+H120N+P131H+Q137E	N204D+Q245S

S9R+A15T+L96LG+H120D+P131S+Q137E	N62D+Q245W+N252E
S9R+A15T+H120N+P131T+N218D	N62D+Q245R+N252V
S9R+A15T+L21LP+T22TV+M119I+N218D+	S9R+A15T+S24P+G61E+A85T+P239S+
Q245I -	Q245A
S9R+A15T+L96LG+H120D+G160D	G102S+M222S+Q245L+N252D
V68A+S106A+G118D+Q245R+T255S+	A15M+V30I+N62D+S99N+L111I+V244A+
L257G+T274L	S265N
S9R+A15T+G61E+A85T+P239L+Q245C	S9R+A15T+T22TG+N62D+V139L+Q245S
S9R+A15T+P131H+S144P	S3T+Q12D+R19W+V30I+S106G+I107M
S9R+A15T+G97GA+Q137E	V68A+A88T+V139L
S9R+A15T+G97GA+H120Q+P131H+Q137E	V51I+L111I+G118D+Q245R
S9R+A15T+L21LW+G100S+V139L+Q245V	V68A+V203L
S9R+A15T+G97GA+Q137H+N218S	A1T+V68A+N116D+G118D
S9R+A15T+L96LG+H120N+P131S+Q137H	V68A+G118D+Q245R
S9R+A15T+G97GA+H120N+Q137E	N62D+V139I+N183D+N185S+V203I+
	Q245R+L262S
S9R+A15T+L96LG+P131T+Q137H	N62D+I72V
S9R+A15T+L96LG+H120N+P131S	N62D+V81A+Q245R
S9R+A15T+V68A+Q137D	T22A+V68A+S106T+G118D
S9R+A15T+G97GA+H120Y+Q137H	V68A+L111I+V203I
S9R+A15T+G97GA+Q137D	G61E+V68A+A169G
S9R+A15T+K94N+H120N+P131H	V68A+L111V
S9R+A15T+L96LG+P131H+Q137D	V68A+G118D+V203A+K251R
S9R+A15T+F50S+H120D+P131H	V68A+G118D
S9R+A15T+G97GA+H120N+Q137D+N248D	A1V+V51A+V68A+V203I
S9R+A15T+L96LG+P131Q+Q137D	V68A+V139L+A223G
<del></del>	

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S9R+A15T+Q245F	S9R+A15T+T22A+N62D
S9R+A15T+Q245S	A98Q+S99D
S9R+A15T+G97GV+H120Q	S9R+P14I+R19K+A98AD
S9R+A15T+G97GA+Q137E+L262V	S9R+A15M+A16P+T22S+S99AD
S9R+A15T+G127E+P131R+Q137H	S99AD+T255R+S256N
S9R+A13V+A15T+I35V+N62D+Q245F	S9R+A15T+T22TQ+S101P
S9R+A15T+Q245V	S9R+A15T+H120R+Q137D+N173S
V139L+Q245F	G97E
S9R+A15T+T22A+V139L+Q245E	Q245W
S9R+A15T+T22L+V139L+Q245V+A254S	S9R+A15T+L96LG+Q137E+Y209H
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N62D+Q245R	Q137D
N62D+A151G	A98AH
V68A+S106T	V51D
S99A+S99SD+V203L	Q12E+P14L+A15T
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	<u>,</u>
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N272V	Q245R
S9R+A15T+G20*+L21F+*61aS+V68A+	S9R+A15T+V68A+H120N+P131S+Q137H+
G160D+Q245R	Q245M

## wherein

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- (a) the variant has protease activity, and
- (b) each position corresponds to a position of the amino acid sequence of subtilisin BPN', shown in SEQ ID NO: 1.
- The protease variant of claim 1, wherein the parent subtilase belongs to the sub-group I-4. S1.
- The protease variant of claim 1, wherein the parent subtilase belongs to the sub-group I-5.. 10 S2, and wherein the parent subtilase preferably is subtilisin 309.
- The protease variant of claim 1, wherein said variant further comprises one or more of 6. the modifications K27R, \*36D, S56P, N62D, V68A, N76D, S87N, G97N, S99SE, S101G, S103A, V104A, V104I, V104N, V104Y, S106A, H120D, H120N, N123S, G159D, Y167A, R170S, R170L, A194P, N204D, V205I, Q206E, L217D, N218S, N218D, M222S, M222A, T224S, A232V, K235L, Q236H, Q245R, N248D, N252K, T274A, S101G+V104N, S87N+S101G+V104N, K27R+V104Y+N123S+T274A, N76D+S103A+V104I, S99D+S101R+ S3T+V4I+S99D+S101R+S103A+V104I+G160S+V199M+V205I+ S103A+V104I+G160S, S3T+V4I+S99D+S101R+S103A+V104I+G160S+A194P+V199M+V205I+L217D, L217D. 20 S3T+V4I+S99D+ S101R+S103A+V104I+G160S+V205I and N76D+V104A.
  - The protease variant of claim 1, comprising the following substitutions: 7. \$101G+\$103A+V104J+G159D+A232V+Q236H+Q245R+N248D+N252K.
- A cleaning or detergent composition, preferably a laundry or dish wash composition, 25 comprising a protease variant of claim 1 and a surfactant.
  - A composition of claim 8, which additionally comprises one or more of an amylase, 9. cellulase, cutinase, esterase, beta-galactosidase, glycoamylase, hemicellulase, lactase, ligninase, lipase, polygalacturonase, and protease.
    - An isolated DNA sequence encoding a protease variant of claim 1 10.
    - An expression vector comprising the isolated DNA sequence of claim 10. 11.

- 12. A microbial host cell transformed with the expression vector of claim 11.
- 13. A microbial host cell of claim 12, which is a bacterium, preferably a *Bacillus*, especially a 5 B. lentus.
  - 14. A microbial host cell of claim 12, which is a fungus or yeast, preferably a filamentous fungus, especially an *Aspergillus*.
- 10 15. A method for producing a protease variant, comprising
  - (a) culturing a host of claim 12 under conditions conducive to the expression and secretion of the variant, and
    - (b) recovering the protease variant.